ORIGINAL ARTICLE

Oral candidal carriage and species identification among betel quid chewers and oral submucous fibrosis patients

Preethi Somashekhar, V. V. Kamath, C. Ramanna

Department of Oral and Maxillofacial Pathology, Dr. Syamala Reddy Dental College, Hospital and Research Institute, Bengaluru, Karnataka, India

Keywords
Betel quid chewing, *Candida albicans, Candida tropicalis*, CHROMagar®, oral submucous fibrosis

Correspondence
Dr. Preethi Somashekhar, Department of Oral and Maxillofacial Pathology, Dr. Syamala Reddy Dental College Hospital and Research Institute, Munnekolala, Marathahalli, Bengaluru - 560 037, Karnataka, India.
E-mail: preeshubham@gmail.

Received 02 May 2016;
Accepted 10 June 2016

doi: 10.15713/ins.jcri.122

Abstract

Background: The *Candida* species are saprophytic component of the normal oral microbiota. The disequilibrium in the homeostasis between *Candida*, host immune system and normal oral bacterial flora promotes a *Candida* carriage.

Aim of the Study: The present study was done to assess oral candidal carriage and species identification among healthy subjects, betel quid chewing (BQC), and oral submucous fibrosis (OSF) patients using CHROMagar® media.

Materials and Methods: Oral swabs collected from the reservoirs of *Candida* species (buccal mucosa and tongue) in study groups using sterile cotton swabs were inoculated on Sabouraud's dextrose agar candidal medium and CHROMagar® *Candida* agar medium consecutively. Determination of *Candida* species was done based on morphology characteristics and pigment produced. The results were statistically analyzed using Chi-square test.

Results: *Candida* carriage was more in samples of buccal mucosa followed by the tongue. *Candida albicans* was the major isolate in both healthy subjects and BQC but with greater frequency in BQC. *Candida tropicalis* was the major isolate in OSF patients predominantly in buccal mucosa followed by the tongue.

Conclusion: *C. tropicalis,* a non-*C. albicans* species, was the major isolate in OSF. This unique finding implicates an immunological change of oral environment brought about by BQC and OSF.

Introduction

The *Candida* genus belongs to a heterogeneous cluster of fungi of the class deuteromycetes.[1] The *Candida* species are saprophytic component of the normal microbiota in gastrointestinal tract, skin, and vagina.[2] In the oral cavity, the *Candida* niches include the tongue followed by palate and buccal mucosa.[3] *Candida* carriage shows variation according to the age and health of an individual. *Candida albicans* is the predominant isolate with a mean carriage of about 18% and 41% in normal individuals and patients, respectively.[4]

The disequilibrium in the homeostasis between *Candida*, host immune system and normal oral bacterial flora favors *Candida* virulence factors such as the ability to form biofilm, various mechanisms to resist host defenses and production of hydrolytic enzymes such as proteases, phospholipases, and hemolysin which cause damage to the tissues.[5] *Candida* causes mycotic infections in both immunocompetent individuals and immunocompromised hosts.[6,7] The increase in the incidence of infections in immunocompromised individuals is due to their greater adaptability to divergent host niches.[8]

In recent years, worldwide, the consumption of tobacco products by youngsters in various forms has increased. The betel quid chewing (BQC) habit is found to be more prevalent in several Asian and South-Asian countries.[9-11] Oral submucous fibrosis (OSF), a chronic potentially malignant disorder, is of multifactorial origin with arecanut chewing as a predominant causative agent.[12] The tobacco contents (nicotine, polycyclic aromatic hydrocarbons, polonium, and nitrosoproline), which constitute as an additive component of betel quid, are reported to provide nutrition for *Candida* and promote their proliferation.[13] They may also increase the colonization of *Candida* by causing an increase in epithelial keratinization, decrease in salivary immunoglobulin A and leukocytic function, and oral epithelial changes such as atrophy, hyperplasia, and dysplasia which may compromise the epithelial integrity.[14]

A shift in the colonization of non-*C. albicans* species and their emergence as major potential pathogens can be related to their virulence factors, the increase in the population at special risk and use of antibiotics.[15] The recognition of *Candida* isolates is of importance in the better understanding of host-pathogen interaction in the pathogenesis of disease and treatment planning. The categorization of *Candida* isolates can be done by various chromogenic media. Chromogenic media are species specific and yield candidal colonies with varying pigmentation due to the formation of secondary substrates by reacting with *Candida* species-specific enzymes. CHROMagar® is a chromogenic media known to identify and differentiate species based on color and colonial characteristics.[16]

This study is focused to assess oral candidal carriage and species identification among healthy subjects, BQC, and OSF patients using CHROMagar® media.

Materials and Methods

The study was done after obtaining permission from the Institutional Review Board. The study participants informed consent was taken before the collection of the sample, and a detailed case history was recorded.

Inclusion criteria

The study participants were in the age group of 20-45 years with equal males and females in the groups (M: F=1:1). They were categorized into three groups.

Group A: Healthy individuals without BQC habit ($n = 5$)
Group B: BQC with a mean duration of BQC habit of 15-25 years and frequency of 5-6 times/day. ($n = 15$)
Group C: OSF with a history of BQC for a mean duration of 15-25 years with a frequency of 5-6 times/day. ($n = 15$).

Exclusion criteria

The study excluded individuals:

- With the concurrent habit of tobacco smoking, alcohol consumption, areca nut and gutka chewing
- Using antifungal agents, antibiotics, non-steroidal anti-inflammatory drugs/steroids within the past 12 weeks
- With systemic disorders such as diabetes mellitus, hepatitis B, hepatitis C, HIV and acquired immunodeficiency syndrome
- Denture wearers.

Sample collection

Oral swabs were collected from the most common oral reservoirs of *Candida* species (buccal mucosa and tongue) using sterile cotton swabs in all study participants.

Candida species identification

The oral swabs were inoculated onto sabouraud's dextrose agar (SDA) (Himedia®) and incubated at 37°C for 48 h. Candidal colonies grown on SDA were inoculated into CHROMagar® *Candida* agar medium. The inoculated plates were incubated at 37°C and observed up to 7 days. *Candida* species recognition was done based on the morphology of the colonies and color produced by the chromogenic reaction between secondary substrates present in chromogenic media containing glucosaminidase substrate and enzymes of *Candida* species. *C. albicans, Candida tropicalis, Candida Krusei,* and *C. glabrata* were identified by the production of green, dark blue, pink, and pink with darker mauve center colored colonies, respectively [Figure 1].[16]

Results

Buccal mucosa

In buccal mucosa of healthy subjects, 60% of the samples showed candidal growth and the only candidal isolate was *C. albicans*.

In BQC, the *Candida* species isolated from the buccal mucosa was as follows: *C. albicans* (66.7%), *C. krusei* (20%), *C. albicans* and *C. krusei* (6.7%), and no colonies (6.7%). *C. albicans* was the major isolate in BQC followed by *C. krusei*.

The *Candida* species isolated in buccal mucosa of OSF patients was as follows *C. tropicalis* (80%), *C. albicans* (13.3%), *C. krusei* (6.7%), *C. albicans,* and *C. krusei* (6.7%). *C. tropicalis* was the major isolate in OSF group followed by *C. albicans* and *C. krusei* [Figure 2 and Table 1].

Tongue

In healthy subjects, 60% of the samples collected from tongue showed candidal growth and the predominant *Candida* species isolated was *C. albicans*. This finding mirrored the buccal mucosa site.

In BQC, the distribution of *Candida* species was as follows: *C. albicans* (26.7%), *C. krusei* (20%), *C. albicans* and *C. krusei* (6.7%), and no colonies (46.7%). *C. albicans* was the major isolate in BQC followed by *C. krusei*.

The *Candida* species isolated in OSF patients was as follows: *C. tropicalis* (60%), *C. albicans* and *C. krusei* (20%), and *C. albicans* (13.3%). *C. tropicalis* was the major isolate in OSF patient followed by *C. albicans* and *C. krusei.* No colonies were isolated in 6.7% of the samples [Figure 3 and Table 2].

Candidal isolate was more in samples of buccal mucosa compared to *Candida* isolates in the tongue. *C. albicans* was the major isolate in healthy subjects and BQC and showed an increase in frequency in BQC compared to healthy subjects.

C. tropicalis was the major isolate in OSF patients in both oral *Candida* reservoirs, predominantly in buccal mucosa followed by tongue. Statistically, the above observations were found to be highly significant. Among 35 total cases, Fisher's exact test value was 29.125 with $P < 0.001$ in buccal mucosa and 22.884 with $P < 0.001$ in tongue.

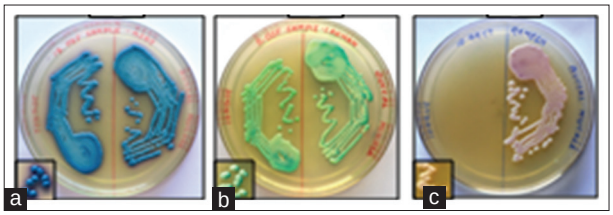

Figure 1: Identification of *Candida* growth on: (a) Sabouraud's dextrose agar, (b) CHROMagar - *Candida tropiclis,* (c) CHROMagar - *Candida albicans,* (d) CHROMagar - *Candida krusei*

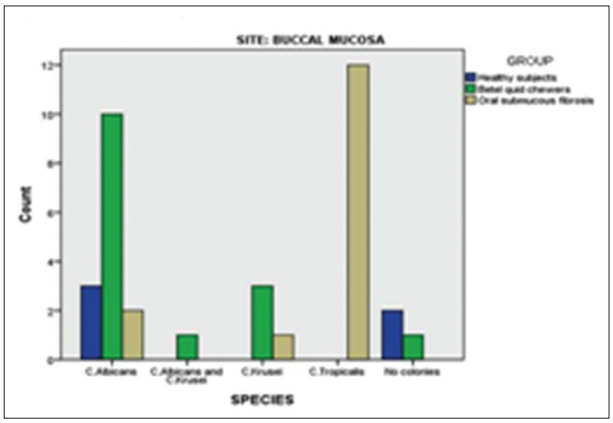

Figure 2: Distribution of *Candida* species on buccal mucosa site in each group

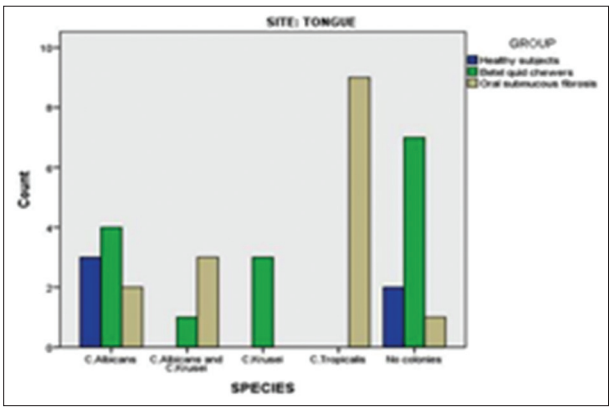

Figure 3: Distribution of *Candida* species on tongue site in each group

Discussion

The homeostasis between *Candida*, host immune system and normal oral bacterial flora determines the role of *Candida* species as either saprophytes or opportunistic pathogens in the oral cavity.[17-20] The prevalence of *Candida* isolates in oral cavity is regulated by endogenous factors such as: (a) Oral epithelial cell antimicrobial peptides such as defensins, cathelicidins and histidine, and epithelial integrity, (b) salivary constituents

Table 1: Distribution of *Candida* species on buccal mucosa site in each group

Candida species distribution in buccal mucosa	Healthy subjects, *n*=5	BQC, *n*=15	Oral submucous fibrosis, *n*=15	Fisher's exact test value	*P* value
C. albicans					
Count	3	10	2	29.125	<0.001
% within group	60.0	66.7	13.3		
C. albicans and *C. krusei*					
Count	0	1	0		
% within group	0.0	6.7	0.0		
C. krusei					
Count	0	3	1		
% within group	0.0	20.0	6.7		
C. tropicalis					
Count	0	0	12		
% within group	0.0	0.0	80.0		
No colonies					
Count	2	1	0		
% within group	40.0	6.7	0.0		
Total					
Count	5	15	15		
% within group	100.0	100.0	100.0		

C. albicans: *Candida albicans*, *C. krusei*: *Candida krusei*, BQC: Betel quid chewers, OSF: Oral submucous fibrosis

such as salivary immunoglobulin A, lysozyme, histidine-rich polypeptides, lactoferrin, and lactoperoxidase, and (c) oral cavity temperature and exogenous factors such as high carbohydrate diet.[21-24] The epithelial cells provide a greater surface area which may promote *Candida* adhesion.[25] The high carbohydrate diet is also reported to facilitate *Candida* adherence to epithelial cells by reduction in pH due to degradation of carbohydrate in saliva.[26] The earlier *in-vitro* studies have reported *C. albicans* to have greater adhesion to oral epithelial cells followed by *C. tropicalis* and *C. parapsilosis.*[27,28] The presence of more α-L-fucose remnants promotes greater adhesion of *C. albicans.*[27] *C. albicans* is major isolate in healthy subjects on the tongue and buccal mucosa in the present study is in consonance with previous observations.

BQ with tobacco chewing is reported to cause an increase in salivary pH of 8-10 due to chemical constituents such as slaked lime and nicotine, a component of tobacco.[29-31] *C. albicans* is able to adapt to pH of 2-10 by actively alkalizing surrounding environment, release of ammonia after amino acid degradation and by phenotypic switching.[32,33] *C. albicans* biofilms are also resistant to neutrophils due to the presence of β-glucans in the extracellular matrix.[34]

Table 2: Distribution of *Candida* species on tongue site in each group

Candida species distribution in tongue	Healthy subjects, *n*=5	BQC, *n*=15	OSF, *n*=15	Fisher's exact test value	*P* value
C. albicans					
Count	3	4	2	22.884	<0.001
% within group	60.0	26.7	13.3		
C. albicans and *C. krusei*					
Count	0	1	3		
% within group	0.0	6.7	20.0		
C. krusei					
Count	0	3	0		
% within group	0.0	20.0	0.0		
C. tropicalis					
Count	0	0	9		
% within group	0.0	0.0	60.0		
No colonies					
Count	2	7	1		
% within group	40.0	46.7	6.7		
Total					
Count	5	15	15		
% within group	100.0	100.0	100.0		

C. albicans: *Candida albicans*, *C. krusei*: *Candida krusei*, BQC: Betel quid chewers, OSF: Oral submucous fibrosis

C. albicans due to their biofilms, greater adherence, and phenotypic switching may show an increased oral carriage in BQC with tobacco in comparison to healthy individuals without BQC habit. In the present study, an increase in the *Candida* carriage in samples isolated from tongue and buccal mucosa of BQC with tobacco was observed in comparison to healthy individuals without BQC. *C. albicans* as the major isolate is similar to previous studies which recorded similar observations on BQC.[10,12,35]

C. tropicalis was isolated as a predominant isolate in OSF patients in samples collected from the tongue and buccal mucosa followed by *C. albicans* and *C. krusei,* which is in contrast to the previous studies. *C. tropicalis* shows the closest genetic similarity to *C. albicans* and exhibits similar phenotypic switching. It is also a major biofilm producer with more hexosamine content in the biofilm which makes it resistant to host immune mechanisms. *C. tropicalis* is also reported to be the predominant isolate in India as per geographic variation.[36-42] This observation is unique to the present study as we could not find similar observations in literature in OSF patients. Similar divergence in mycotic flora has been reported in few populations which can be attributed to the influence of nutritional, social, environmental factors, and oral hygiene practices.[39,43,44]

Interestingly, Reichart *et al.* reported that betel quid contents might not influence *Candida* species.[10] The present observation of the switch in the *Candida* species, however, does not bear out this observation. It is most likely that development of OSF alters the oral environment both in the tissue and salivary component sufficient to induce the growth of *C. tropicalis*. Incidentally, the latter is known to proliferate in conditions of immunosuppression in the human body. Whether an element of immunosuppression known to exist in OSF was contributory to the development of the *C. tropicalis* is debatable. Nevertheless, the statistical significance of the predominance of *C. tropicalis* alludes to the change in the oral environment caused by the BQC and the subsequent development of OSF.

Cohabitation of *Candida* is well documented. In the present study, we isolated two cohabitating species (*C. albicans* and *C. krusei*) in one buccal mucosa sample and 3 tongue samples out of 15 OSF patients. In general, an individual has species-specific niches and harbors a specific species.[36] An isolation of two species is reported in few individuals; such findings are more commonly reported in hospitalized and immunocompromised individuals.[27,45]

Conclusion

An emergence of *C. tropicalis* as a major isolate in OSF is a unique finding in this study which may suggest a shift of *Candida* isolates to non-*C. albicans* species mainly due to host environment variation. The singular observation of this change in OSF patients is interesting and needs further corroboration. It necessitates further studies among these study groups with large sample size, species-specific confirmatory tests which may be beneficial in recognition of *Candida* species and treatment planning of OSF patients.

References

1. Akpan A, Morgan R. Oral candidiasis. Postgrad Med J 2002;78:455-9.
2. Shao LC, Sheng CQ, Zhang WN. Recent advances in the study of antifungal lead compounds with new chemical scaffolds. Yao Xue Xue Bao = Acta Pharm Sin 2007;42:1129-36.
3. Epstein JB, Pearsall NN, Truelove EL. Quantitative relationships between *Candida albicans* in saliva and the clinical status of human subjects. J Clin Microbiol 1980;12:475-6.
4. Odds FC. Ecology of *Candida* and epidemiology of candidosis. *Candida* and Candidosis. London: Bailliere Tindall; 1988. p. 68-92.
5. Silva S, Negri M, Henriques M, Oliveira R, Williams DW, Azeredo J. *Candida glabrata*, *Candida parapsilosis* and *Candida tropicalis*: Biology, epidemiology, pathogenicity and antifungal resistance. FEMS Microbiol Rev 2012;36:288-305.
6. Arendorf TM, Walker DM. The prevalence and intra-oral distribution of *Candida albicans* in man. Arch Oral Biol 1980;25:1-10.

7. Reichart PA, Samaranayake LP, Philipsen HP. Pathology and clinical correlates in oral candidiasis and its variants: A review. Oral Dis 2000;6:85-91.
8. Pfaller MA, Diekema DJ, Procop GW, Rinaldi MG. Multicenter comparison of the VITEK 2 antifungal susceptibility test with the CLSI broth microdilution reference method for testing amphotericin B, flucytosine, and voriconazole against *Candida* spp. J Clin Microbiol 2007;45:3522-8.
9. Javed F, Tenenbaum HC, Nogueira-Filho G, Qayyum F, Correa FO, Al-Hezaimi K, *et al.* Severity of periodontal disease in individuals chewing betel quid with and without tobacco. Am J Med Sci 2013;346:273-8.
10. Reichart PA, Khongkhunthian P, Samaranayake LP, Yau J, Patanaporn V, Scheifele C. Oral *Candida* species and betel quid-associated oral lesions in Padaung women of Northern Thailand. Mycoses 2005;48:132-6.
11. Reichart PA, Schmidtberg W, Samaranayake LP, Scheifele C. Betel quid-associated oral lesions and oral *Candida* species in a female Cambodian cohort. J Oral Pathol Med 2002;31:468-72.
12. Hsia CC, Sun TT, Wang YY, Anderson LM, Armstrong D, Good RA. Enhancement of formation of the esophageal carcinogen benzylmethylnitrosamine from its precursors by *Candida albicans*. Proc Natl Acad Sci U S A 1981;78:1878-81.
13. Samaranayake LP, MacFarlane TW. Host factors and oral candidosis. Oral candidosis. London: Wright; 1990. p. 66-103.
14. Sudarshan R, Annigeri RG, Vijayabala SG. Pathogenesis of oral submucous fibrosis: The past and current concepts. Int J Oral Maxillofac Pathol 2012;3:27-36.
15. Horn DL, Neofytos D, Anaissie EJ, Fishman JA, Steinbach WJ, Olyaei AJ, *et al.* Epidemiology and outcomes of candidemia in 2019 patients: Data from the prospective antifungal therapy alliance registry. Clin Infect Dis 2009;48:1695-703.
16. Manikandan C, Amsath A. Isolation and rapid identification of *Candida* species from the oral cavity. Int J Pure Appl Biosci 2013;1:23-7.
17. Luo S, Skerka C, Kurzai O, Zipfel PF. Complement and innate immune evasion strategies of the human pathogenic fungus *Candida albicans*. Mol Immunol 2013;56:161-9.
18. Yan L, Yang C, Tang J. Disruption of the intestinal mucosal barrier in *Candida albicans* infections. Microbiol Res 2013;168:389-95.
19. Mayer FL, Wilson D, Hube B. *Candida albicans* pathogenicity mechanisms. Virulence 2013;4:119-28.
20. Mech F, Wilson D, Lehnert T, Hube B, Thilo Figge M. Epithelial invasion outcompetes hypha development during *Candida albicans* infection as revealed by an image-based systems biology approach. Cytometry A 2014;85:126-39.
21. Pereira-Cenci T, Del Bel Cury AA, Crielaard W, Ten Cate JM. Development of *Candida*-associated denture stomatitis: New insights. J Appl Oral Sci 2008;16:86-94.
22. Scully C. Oral and Maxillofacial Medicine. Edinburgh: Wright; 2004. p. 252-7.
23. Brassart D, Woltz A, Golliard M, Neeser JR. *In vitro* inhibition of adhesion of *Candida albicans* clinical isolates to human buccal epithelial cells by Fuc alpha 1----2Gal beta-bearing complex carbohydrates. Infect Immun 1991;59:1605-13.
24. Ghannoum MA, Burns GR, Elteen KA, Radwan SS. Experimental evidence for the role of lipids in adherence of *Candida* spp. To human buccal epithelial cells. Infect Immun 1986;54:189-93.
25. Douglas LJ. Surface composition and adhesion of *Candida albicans.* Biochem Soc Trans 1985;13:982-4.
26. Dalle F, Wächtler B, L'Ollivier C, Holland G, Bannert N, Wilson D, *et al*. Cellular interactions of *Candida albicans* with human oral epithelial cells and enterocytes. Cell Microbiol 2010;12:248-71.
27. Henriques M, Azeredo J, Oliveira R. *Candida* species adhesion to oral epithelium: Factors involved and experimental methodology used. Crit Rev Microbiol 2006;32:217-26.
28. Lima-Neto RG, Beltrão EI, Oliveira PC, Neves RP. Adherence of *Candida albicans* and *Candida parapsilosis* to epithelial cells correlates with fungal cell surface carbohydrates. Mycoses 2011;54:23-9.
29. Anu RR. Biochemical changes in betel quid chewers and non-chewers of Kakkarai, Thanjavur district of Tamil Nadu. Asian J Pharm Clin Res 2015;8:122-4.
30. Thomas SJ, MacLennan R. Slaked lime and betel nut cancer in Papua New Guinea. Lancet 1992;340:577-8.
31. Tilakaratne WM, Klinikowski MF, Saku T, Peters TJ, Warnakulasuriya S. Oral submucous fibrosis: Review on aetiology and pathogenesis. Oral Oncol 2006;42:561-8.
32. De Bernardis F, Mühlschlegel FA, Cassone A, Fonzi WA. The pH of the host niche controls gene expression in and virulence of *Candida albicans*. Infect Immun 1998;66:3317-25.
33. Vylkova S, Carman AJ, Danhof HA, Collette JR, Zhou H, Lorenz MC. The fungal pathogen *Candida albicans* autoinduces hyphal morphogenesis by raising extracellular pH. MBio 2011;2:e00055-11.
34. Xie Z, Thompson A, Sobue T, Kashleva H, Xu H, Vasilakos J, *et al*. *Candida albicans* biofilms do not trigger reactive oxygen species and evade neutrophil killing. J Infect Dis 2012;206:1936-45.
35. Javed F, Yakob M, Ahmed HB, Al-Hezaimi K, Samaranayake LP. Oral *Candida* carriage among individuals chewing betel-quid with and without tobacco. Oral Surg Oral Med Oral Pathol Oral Radiol 2013;116:427-32.
36. Anila K, Hallikeri K, Shubhada C, Naikmasur VG, Kulkarni RD. Comparative study of *Candida* in oral submucous fibrosis and healthy individuals. Rev Odonto Ciência 2011;26:71-6.
37. Ariyawardana A, Panagoda GJ, Fernando HN, Ellepola AN, Tilakaratne WM, Samaranayake LP. Oral submucous fibrosis and oral yeast carriage - A case control study in Sri Lankan patients. Mycoses 2007;50:116-20.
38. Kamat MS, Vanaki SS, Puranik RS, Puranik SR, Kaur R. Oral *Candida* carriage, quantification, and species characterization in oral submucous fibrosis patients and healthy individuals. J Investig Clin Dent 2011;2:275-9.
39. Samaranayake LP. Host factors and oral candidosis. In: Samaranayake LP, Mac Farlane TW, editors. London: Butterworth & Company Ltd.; 1990. p. 75-6.
40. Mohandas V, Ballal M. Distribution of *Candida* species in different clinical samples and their virulence: Biofilm formation, proteinase and phospholipase production: A study on hospitalized patients in Southern India. J Glob Infect Dis 2011;3:4-8.
41. Dag I, Kiraz N, Oz Y. Evaluation of different detection methods of biofilm formation in clinical *Candida* isolates. Afr J Microbiol Res 2010;4:2763-8.
42. Vinitha M, Ballal M. Biofilm as virulence marker in *Candida*

isolated from blood. World J Med Sci 2007;2:46-8.
43. Reichart PA, Samaranayake LP, Samaranayake YH, Grote M, Pow E, Cheung B. High oral prevalence of *Candida krusei* in leprosy patients in northern Thailand. J Clin Microbiol 2002;40:4479-85.
44. Xu J, Mitchell TG. Geographical differences in human oral yeast flora. Clin Infect Dis 2003;36:221-4.
45. Krogh P, Holmstrup P, Thorn JJ, Vedtofte P, Pindborg JJ. Yeast species and biotypes associated with oral leukoplakia and lichen planus. Oral Surg Oral Med Oral Pathol 1987;63:48-54.

How to cite this article: Somashekhar P, Kamath VV, Ramanna C. Oral candidal carriage and species identification among betel quid chewers and oral submucous fibrosis patients. J Adv Clin Res Insights 2016;3:133-138.